FURTHER STUDIES OF GRANULAR DEGENERATION OF THE ERYTHROCYTE.[1]

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IN a previous communication, read at the meeting of the Association of American Physicians, held at Washington, April 29, 1901, and published in THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES,[2] we presented our investigations of granular degeneration of the erythrocyte and in particular the facts bearing on the relations of this condition to lead poisoning. The material which we then accumulated gave us the opportunity to discuss the morphology of the condition and also its probable etiology. The direct dependence of the degeneration upon lead poisoning was shown by the regular occurrence of the granules in cases of lead poisoning admitted to the hospital, by the invariable discovery of the granules in men working in various capacities in lead works, by the discovery of granules soon after the administration of therapeutic doses in healthy individuals, and by the typical appearance of the corpuscles in dogs subjected to subacute or chronic lead poisoning. Further, we feel prepared to express a positive belief that the granular degeneration is the result of protoplasmic changes rather than the consequence of a nuclear fragmentation. We could see no relation between the granular condition and polychromatophilia excepting a frequent coincidence of the two conditions. Our belief was and remains that this granular condition is a true degeneration of an independent kind. We did not at that time feel prepared to state whether it occurs in lead poisoning as a result of the direct action of lead, or whether it is the consequence of anæmic conditions occasioned by the lead; nor did we then arrive at any conclusion as to the place of origin of the changes found in the circulating erythrocyte. The present communication has for its purpose: in the first place a further discussion of the probable nature of the granules, and in the second place the report of some observations made to determine, if possible, the location in which the changes take place.

To determine the place of origin of the changes, we studied the blood taken from different parts of the circulation as well as scrapings and preparations from the bone marrow and splenic pulp. The material was taken from four animals that had been kept under the influence of lead for a considerable length of time. First, blood was taken from an artery and vein of the stomach, intestines, liver, spleen, and heart of etherized animals two hours after they had been given large doses of acetate of lead. The animals in each case had been under the influence of lead for some time, and granular erythrocytes were present in the circulating blood. The purpose of this experiment, which was prompted by Grawitz's view regarding the gastro-intestinal origin of the granular degeneration, was to determine whether erythrocytes showing this degeneration occur more abundantly in the blood of the portal vein and its tributary circulations than elsewhere. In only one of our experiments could we recognize an unequal distribution of granular cells. In this case more granular erythrocytes were found in the veins of the spleen, liver, and intestines than elsewhere. We, of course, cannot pretend that this single experience is of much weight in deciding the matter under discussion.

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[2] September, 1901.

The bone marrow and spleen were examined in two ways: (*a*) By preparing spreads made from scrapings of the cut surface of the organs, and (*b*) by embedding portions of the organs, hardened in various ways, in paraffin, and sectioning. In the spreads granular erythrocytes, both nucleated and non-nucleated, were easily demonstrated, but were not more abundant than in the circulating blood. Fixation by heat and in mixtures of ether and absolute alcohol and in absolute alcohol and 1 per cent. formol gave the best results, heat being by far the most satisfactory. The sections did not in any case show granular cells, as the red corpuscles were more or less crenated, whatever the method of fixation. We could not, therefore, be certain of the presence or absence of granular erythrocytes.

The result of these experiments then gave us no indications of the probable place of origin of the granular degeneration. In no instance did we find more abundant granular cells in the bone marrow or spleen than in the peripheral blood.

Some experiments seemed desirable to determine whether the granular cells existed in the circulating blood or whether, on the other hand, they are artefacts. The characters of the granules, the regularity of their occurrence in the blood in certain diseases, as well as their discovery in scrapings of tissues had convinced us that the granular corpuscles are not artefacts, and the question has not been seriously raised. Nevertheless, we thought it advisable to practice injection of certain stains to determine whether the fresh blood-corpuscles after such injections would show granular cells. Dogs that had been kept under the influence of lead until the blood showed granular erythrocytes in abundance were etherized, and solutions of methylene blue or neutral-roth in 0.8 per cent. NaCl solution were injected into a vein. Specimens of blood were taken at various intervals of time from the ear and examined fresh or after drying and fixation. The injections of methylene blue gave the only positive results. The blood in the successful cases showed nucleated and non-nucleated erythrocytes containing pale blue granules. The appearances of these granular cells were in every respect identical with those of the granular cells seen in stained spreads of the blood obtained before the injection of methylene blue, excepting that the staining of the granules was a little less intense. We could not confirm the *observation of* Sabrazes, Bourret, and Legér *that the granules are eccentrically* situated and show a tendency to extrusion.

The dried specimens of blood prepared as above described showed exactly similar conditions.

We have made a few attempts at artificial production of granular degeneration outside the body, and refer to them here, though we are aware of their unsatisfactory character. Quantities of human blood and of dog's blood were mixed with blood serum of dogs that had been under the influence of lead for some time. In no case did we reach positive results. The rapid disintegration of the red corpuscles under such circumstances naturally renders the experiment of little practical value. Equally unsatisfactory results attended the mixture of human blood and dog's blood with various solutions of acetate of lead. Sabrazes, Bourret, and Legér attempted to produce the degeneration by injecting solutions of lead into a ligated vein of a dog's leg, but without success.

A study of the various pathological conditions in which granular degeneration of the erythrocytes occurs seemed to us of possible importance in elucidating the question of the place of origin of the degeneration, and perhaps also its nature. Such a study is of course of even greater and more immediate interest in determining the diagnostic value of this reaction of lead poisoning. We therefore studied a considerable series of cases representing various diseases, and will refer to our results under the headings—chlorosis, pernicious anæmia, leukæmia, and miscellaneous medical and surgical diseases. The results obtained show that no explanation of the nature of the degeneration or its place of origin could be reached in this way.

CHLOROSIS. Contrary to the experience of other observers, we have found granular erythrocytes quite *frequently in this disease. Among* 18 cases examined by us there were 11 *in which the* granular cells were abundant. In 6 of the 11, the granules were fine; in the other 5 they were fine and occasionally coarse. In only 1 of the cases were the granules numerous. In this case the hæmoglobin was only reduced to 50 per cent., while in most of the cases a far greater reduction was noted. In all of the 11 cases polychromatic cells were seen, but these seemed to bear no relation in intensity or number to the granular cells.

PERNICIOUS ANÆMIA. Judging from the seven cases which we observed, and from the reports published by others, granular degeneration is a constant condition in advanced stages of pernicious anæmia. We found the granules fairly numerous and at times very large and conspicuous. Though, however, in the number and the character of the granules the blood of pernicious anæmia was usually distinguished from that of other diseases, we did not often find in pernicious anæmia the extraordinary number and variety of granules, coarse and fine seen in nearly all of the cases of lead intoxication which we have examined; and the other characters of the blood would render a differentiation easy.

LEUKÆMIA. We examined ten cases of this disease and found granules in every one, though they were usually fine and not present in large numbers. We shall have occasion to refer again to this matter, as the occurrence of the granules in leukæmia is of particular importance in connection with the determination of the nature of granular degeneration, nucleated erythrocytes being so abundant in this disease and nuclear degenerations of all kinds so frequent. We should look then to this disease more than in any other for a confirmation of the view that granular degeneration results from fragmentations of the nucleus.

MISCELLANEOUS MEDICAL AND SURGICAL DISEASES. In addition to the 35 cases above referred to, we have examined the blood in 105 patients in the medical and surgical wards of the University Hospital, and have discovered granular erythrocytes in 34 of these cases. In the 34 cases the diagnoses were as follows: typhoid fever, 3 cases; valvular heart disease, 3; peritonitis, 3; septicæmia, 3; tuberculous arthritis, 2; malaria, 2, and pertussis, heart disease and nephritis, lobar pneumonia and pleurisy, phthisis, malignant endocarditis, aneurism and nephritis, nephritis and anæmia, splenic anæmia, secondary anæmia, pseudoleukæmia, chronic diarrhœa, phlyctenular conjunctivitis, orchitis, carcinoma of stomach, lymphoma of neck, sarcoma of neck, empyema, osteomyelitis, each 1 case.

Several observers have reported the discovery of granular red cells in cases of sepsis, and, though the direct bearing of septic infections upon the conditions has not been established, it is of interest to note that this condition was present in some of the cases enumerated above. Perhaps half of the above 34 cases were more or less septic. In the other half of the cases there was no such element, but the high grade of anæmia may have been alone operative in these. In several of the cases no clue whatever to the causation of the granules could be found. We might speak of them as accidental occurrences, but this would not serve as an explanation. In none of the 34 miscellaneous cases were the granules abundant or conspicuous. Two instances in which the blood picture approached that found in lead poisoning were a case of phlyctenular conjunctivitis in a boy, and a case of chronic diarrhœa in a man who had possibly taken acetate of lead in the treatment of his condition before he came under our observation. Even in these two cases we could easily distinguish the blood from that of lead poisoning, and in the remaining 32 cases the comparative sparseness of the granules and their finer character made the distinction very easy.

There were 71 cases in which we did not find granules. Some of these may be mentioned for the sake of showing that the classes of diseases in which the granules occur or do not occur give no indication of the probable source of the granular cells; and also to show that the condition of the blood as to infection or anæmia will not alone explain the degeneration.

We failed to find any granules in the following cases: 5 cases of valvular heart disease, 4 of phthisis, 4 of appendicitis, 7 of typhoid fever, 5 of sepsis, 6 of grippe, 3 of carcinoma of the stomach, 3 of diabetes, 2 of pneumonia, and in one case each of malaria, splenic anæmia, glanders, actinomycosis, and tetanus.

With the single exception of lead poisoning, no condition that we know of regularly causes this change, though other conditions operate to this end in occasional cases. As far as lead is concerned, we may refer again to our previous studies. In each of seven clinical cases of plumbism we found enormous numbers of the granular cells; and we may add six recent instances. In twenty-one men working in lead works, but showing no symptoms of lead poisoning, the granules were always found, but in varying numbers. Two of these men had worked in the lead works only four days. Moritz found the granular cells in six men working in lead works, but having no symptoms; and other observers, as Strauss, Grawitz, Behrendt, Sabrazes, Bourret, Legér, and Hamel (the last in 25 cases) found the granules in cases of lead poisoning. Strauss also discovered them in atropine poisoning and in rabbits and frogs poisoned with pyrodine. Kaminer and Rohnstein found granular cells in the blood of rabbits poisoned with phenylhydrazin on the fourteenth day of the intoxication. Sabrazes, Bourret, and Legér refer to a case of fatal copper poisoning in which numerous granules were found, but they failed to produce the degeneration in guinea-pigs by intraperitoneal injections of various toxic and non-toxic substances, such as distilled water, thallium acetate, carbonate of lithium, and sulphate of atropine, or by inhalations of nitrite of amyl, pyrodine, and phenyl hydrazin. Injections of lead acetate, however, caused the degeneration very rapidly in guinea-pigs as well as in rabbits. In our own experiments we have found the degenerations after poisoning a dog with potassium chlorate; the granules did not appear until shortly before death, but became very numerous. Another dog slowly intoxicated with corrosive sublimate showed only a few granules shortly before death. They were also present in large numbers in the blood of dogs kept under the influence of toxic doses of pyrodine for many days. It is evident, therefore, that toxic causes of various sorts are capable of producing the granular degeneration we are studying, but it is equally evident that no poison thus far studied is as regular in its production of the degeneration or as prompt in its action as is lead.

The discussion of the nature of the granular condition of the red corpuscles has divided observers into two groups: those holding that the granules originate from the nucleus, and those who regard them as the product of a specific degeneration of the protoplasm.

The former view was the one first put forward after the granular cells were recognized, and has been lately defended by Askanazy, Lazarus, Engel, Litten and Strauss. These authors look upon the granules as the result of nuclear fragmentation—karyorrhexis—and they believe that it is the means by which the nucleated cell normally loses its nucleus before becoming an adult cell and entering in the peripheral circulation. The attractiveness of this theory is largely dependent upon its adaptability as an explanation of the disappearance of the nucleus, a matter which has remained unsolved in spite of painstaking examinations of histologists; but more definite facts are required to establish the truthfulness of the theory. It would seem entirely reasonable to expect blood pictures showing a gradual transition from the state of normal nucleated cells to that of erythrocytes containing scattered granules, but without a nucleus. As a matter of fact, Litten claims that he has seen direct transitions from the normal nucleus to fully granular cells, both in the circulating blood and in the bone marrow, and he states that the nucleus gradually becomes paler as the granules increase in number and size. Somewhat similar statements are made by Ewing. Litten's observations were made in only one case, a rapidly fatal one of pernicious anæmia. Our own observations have included a large number of cases of intense anæmia and of various intoxications, both clinical and experimental, and we have found in these abundant granular cells in the circulating blood as well as in the bone marrow and other organs, but we have never in a single instance among the thousands of granular cells that we have carefully examined found the slightest suggestion of a nuclear fragmentation productive of granulations. We have repeatedly found and figured, from paintings by an artist and from photographs, associations of nuclei and granules, that is, we have recorded the occurrence of granules in nucleated cells; but in every instance the periphery of the nucleus has either been clear-cut as in the normal nucleated cell, and the chromatin of the nucleus has presented normal appearances, or when degeneration has been present there has been nothing suggesting even remotely an origin of granules in degeneration of the nucleus. The conditions in the blood of leukæmia seems to us to be particularly important in this connection. Nucleated erythrocytes with and without granules were very abundant in some of the cases studied, and in the same cases we often found evidences of nuclear degenerations, both in the white and in the red corpuscles. In no instance, however, could we see evidence of a transition of nucleated cells to granular cells.

If the theory of the gradual fragmentation of the nucleus were established, we should expect the evidences of such fragmentation to be most abundant in the bone marrow, where nucleated cells become non-nucleated; but our experience has been that the nucleated cells and the granular cells in bone marrow have been exactly the same as those of the peripheral circulation.

An observation made by us in several instances of still greater significance to disprove the nuclear origin of the granules was the discovery of karyomitosis in granular erythrocytes. One observation of this sort seems to us more valuable than any amount of negative evidence or of theorizing. It is unlikely that a nucleus could at the same time be in a state of active mitosis and of karyorrhexis.

The second theory regarding the nature of the degeneration, and the one to which we incline, is that which has been defended by Grawitz, Moritz, Hamel, and Bloch, and which attributes the granular condition to a peculiar protoplasmic degeneration. In the consideration of this theory we would first of all direct attention to the fact that all of the protoplasmic degenerations of erythrocytes that have been thus far described are characterized by the development of a basophilic tendency. Thus, in the studies of Maragliano and Castellino particular attention was directed to this peculiarity of the degenerated areas, and the behavior of polychromatophilic cells, so generally considered degenerative, is very similar. In no case is there the distinct basic affinity of the degenerated parts of the cell that is seen in the granular degeneration under consideration, but this is a point of too little importance to disprove our view.

Grawitz did not consider the granules the results of nuclear fragmentation, because in his earlier work he had not seen these granules in nucleated red corpuscles. He had expected to find them if these granulated cells were the unfinished red cells prematurely placed in the peripheral circulation. Under such circumstances granular nucleated red corpuscles should be found, or at least transitional forms should occur. Later he found nucleated erythrocytes with these granules in the protoplasm, but the nucleus was to all appearances normal. This last observation induced him to study the blood-forming organs, but here he was unable to see any evidence of these granules. From these observations he came to the conclusion that the granules are the result of degenerative processes in the hæmoglobin, caused by poisons acting directly upon the erythrocytes, and suggests that this takes place outside of the blood-forming organs.

Our own studies have shown granular nucleated erythrocytes in the peripheral blood, and also granular erythrocytes, nucleated and non-nucleated, in the bone marrow; but, like Grawitz, we have failed to find any transitions from the non-granular nucleated erythrocytes to the granular non-nucleated cell. While the proof of the protoplasmic origin of the granules is therefore more or less negative or indirect, we believe the evidence against the nuclear origin of the granules is direct and positive. This evidence may be summarized as follows:

1. Karolytic and karyorrhexic changes may be observed in nucleated red cells without showing any granular change in the protoplasm of the cells; on the other hand, granular degeneration may accompany these nuclear changes without association of the nuclear and granular processes.

2. The granulated red cells (coarse or fine granules) never show the remains or a suggestion of a former nucleus.

3. The granules are observed in karyokinetic red cells, and we have seen them associated with the several stages of the dividing nucleus. We cannot believe that such a progressive and retrogressive change can be present in the nucleus at the same time, without internal evidence of degeneration.

4. The very early appearance of these granules in the blood taken from the peripheral circulation (twenty-five hours after a dose of 7½ grains of the acetate of lead has been taken by one of us) to a certain extent indicates a probable beginning of the destructive changes in the erythrocytes (non-nucleated) of the peripheral blood, rather than in the erythrocytes (nucleated) at the moment in process of formation in blood-making organs.

5. The granules observed in the bone marrow were absolutely the same as those seen in the peripheral blood. Those in the nucleated cells, chiefly normoblasts, showed no evidence of derivation from the nucleus, the nuclei being in each case normal in size, shape, and staining qualities and like those of the neighboring nucleated cells which did not contain granules.

6. Finally it seems to us that in certain cases of leukæmia in which great numbers of nucleated red cells are always present, if these granules were nuclear derivatives, distinct steps or transitions could be demonstrated. Such is not the case. On the contrary, distinct degenerative changes, karyorrhexis, karyolysis, pyknosis, atrophy of the nucleus, etc., are present sometimes with and sometimes without granular protoplasm; but there are never in our experience any

transitional stages to indicate gradual destruction of nuclei with liberation of substance that has gone to form granules. In addition very many of the nucleated cells showing nuclear degeneration contained no granules.

REFERENCES.

Askanazy. Zeitschr. f. klin. Med., Band xxiii., 1893.
Lazarus. 1. Die Anaemie. Nothnagel series. 2. Verhandl. des Vereins f. Inner. Med., Deutsch. med. Woch., No. 23, 1896.
Litten. Verein-beilage d. Deutsch. med. Woch., No. 16, November 30, 1899.
Strauss. Ibid.
Bloch. Ibid.
Grawitz. Deutsch. med. Woch., No. 36, 1899. Berlin. klin. Woch., Nos. 9 and 20, 1900.
C. S. Engel. Virchow's Archiv, Band ciii.
Hamel. Deutsch. Archiv f. klin. Med., 67, 1900.
Behrendt. Deutsch. med. Woch., No. 44, 1899.
Sabrazes, Bourret, and Léger. Journ. de Physiol. et de Pathol generale, November 6, 1900.
Kaminer and Rohnstein. Berlin. klin. Woch., No. 31, 1900.
Moritz. Deutsch. med. Woch., January 24 and 31, 1901.

THE LATERAL CHAIN THEORY OF EHRLICH AS EXPLANATORY OF TOXINS, ANTITOXINS, BACTERIOLYSINS, AND HÆMOLYSINS.

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THE last three years of Continental activity have added so much to our knowledge of the blood in both normal and immunized animals that a retrospect and conclusions by the one who has been most closely identified with progress along these lines is particularly grateful. Ehrlich in several recent communications has reiterated his "lateral or side chain" theory as explanatory of toxins and antitoxins, and found new data in his and others' works on bacteriolysins and hæmolysins from which he has enlarged and made more conclusive this admirable theory of cell mechanism.

TOXINS AND ANTITOXINS. Toxins are labile, poisonous substances, elaborated by plant and animal cells, of which we have no exact chemical knowledge, but whose characteristics are recognizable by their physiological effect on animals. Toxins differ from all known chemical poisons in these important particulars: 1. They are subject to cell assimilation, that is, they enter into direct chemical combination with protoplasm, whereas chemical substances either destroy the cell or are present in it in solution. 2. Toxins can call forth the production of antitoxins in the animal body, while chemical poisons cannot. 3. The action of toxins is delayed by a considerable incubation period.